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ATTEMPTS TO CULTIVATE THE BACTEROIDS OF THE BLATTIDAE.[1]

MARSHALL HERTIG.

Since the discovery by Błochmann in 1887 of the bacteroids in the fat-body and eggs of cockroaches, these organisms have been studied by a number of investigators. The bacteroids are organisms very closely resembling bacteria in their morphology and staining reactions. They are found in the fat-body of roaches, filling the cytoplasm of certain cells, the bacteriocytes. They are also found forming a layer over the surface of ovarian eggs and in the yolk of the developing egg. They migrate from the yolk into the fat-cells of the half-grown embryo, where they are found in older embryos, nymphs and adults. The bacteroids have been found in all individuals of nine species of Blattidæ thus far studied, namely, *Blatella germanica*, *Blatta orientalis*, *Ectobia livida*, *Ectobia lapponica*, *Blaberus* sp., *Periplaneta americana*, *Periplaneta australasiæ*, *Parcoblatta virginica* and *Parcoblatta pennsylvanica*. No blattid is known to lack the bacteroids.

The morphology and behavior of the bacteroids in the host tissues are almost identical in all the species studied. The relation of the bacteroids to the host is not understood. The relationship may be merely one of harmless parasitism, there being maintained a delicately adjusted equilibrium between host and parasite, or it may be one of symbiosis, in which both bacteroids and their carriers derive some benefit from the association.

The striking morphological resemblance of the bacteroids to ordinary bacteria and the fact that they multiply within the body of the cockroach have led a number of workers to attempt

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their culture in artificial media. Blochmann, their discoverer, was the first to attempt their culture in 1887. He employed a number of common laboratory media, as well as a special cockroach-infusion medium, but obtained only negative results. A few years later, Krassilschtschik ('89), according to Cholodkowsky ('91), also attempted the artificial culture of the blattid bacteroids in connection with his work on certain bacteria ("biophytes") found in the bodies of aphids. Though successful in the cultivation of the aphid bacteria, Krassilschtschik failed in his efforts to cultivate the blattid bacteroids. Forbes ('92), in his work on the bacteria normal to the digestive tract of the Hemiptera, stated that he had been unable to cultivate the bacteria found in the fat-bodies of cockroaches. However, Mercier ('06, '07) announced that he had succeeded in obtaining in pure culture on routine media, the bacteroids of *Blatta orientalis*. Tubes of bouillon were inoculated with material withdrawn by means of a sterile pipette from the oötheca, one end of which had been sterilized by flaming. In forty such experiments Mercier obtained pure cultures of an aërobic, motile spore-bearer, which he named *Bacillus cuenoti*. This organism, which grew luxuriantly on all the common laboratory media, resembled closely in cultural characteristics *Bacillus subtilis* and *B. mesentericus*, organisms commonly occurring as contaminators in cultures. Mercier satisfied himself that *Bacillus cuenoti* represented the cultural form of the bacteroids and made certain morphological correlations between the bacteroids and *Bacillus cuenoti*. Mercier obtained cultures from only the single stage, the oötheca, of but one species, *Blatta orientalis*. Javelly ('14) was unable to confirm Mercier's results. Cultures made by him according to Mercier's technique from a number of oöthecæ of *Blatta orientalis* and *Blatella germanica* remained sterile. Javelly concluded that *Bacillus cuenoti* was merely contamination, and that the bacteroids had not been cultivated in artificial media. Quite recently Glaser ('20) has reported the artifical culture of the bacteroids of *Periplaneta americana* and *Parcoblatta virginica*. The organisms obtained by him from both species are spirilla growing readily on routine media. These organisms differ in many important respects from *Bacillus cuenoti*.

In the work of the writer on the bacteroids of the Blattidæ, the results of which are in manuscript, a number of attempts have been made to cultivate artificially the bacteroids of *Blatella germanica*, *Blatta orientalis* and *Periplaneta americana*. The culture media employed include bouillon, agar, gelatin, sugar bouillon (under aërobic and anaërobic conditions), rabbit blood agar, peptone and ascitic fluid-bouillon. In obtaining the bacteroids for inoculation the following technique was employed. Oöthecæ were flamed at one end, a sterile capillary pipette thrust through such flamed surface to the opposite end of the oötheca, thus withdrawing material from within the unflamed as well as the flamed end. This material, which was shown by repeated examinations to contain bacteroids, was transferred to tubes of media. Cultures were also inoculated with bacteroids obtained from the fat-body and ovary. Segments of the abdomen were gently pulled apart by means of forceps, the intersegmental membrane being ruptured. By manipulating the forceps in pulling, one of the segments could be lifted away from the viscera, so that when the rupture occurred the segment would project like a shelf above the digestive tract with its adhering masses of fat-body. With a sterile platinum needle, portions of fat-body were "fished" from the under side of this shelf or from the surface of the digestive tract, care being taken not to touch the torn edge of the segment. By this method remarkably little contamination was encountered. Many cultures remained sterile, or yielded but a few scattered colonies on solid media.

From 20 oöthecæ of *Blatella germanica*, 25 cultures were made. 16 of these 25 remained sterile, while in the other nine there appeared a variety of organisms, none of which resembled the organisms obtained by either Mercier or Glaser. Comparable results were obtained in cultures from the fat-body and ovary. A number remained sterile, while in the other cultures appeared a variety of contaminating organisms. Attempts to cultivate the bacteroids of *Blatta orientalis* and *Periplaneta americana* yielded results entirely similar to those obtained with *Blatella germanica*.

In the endeavor to eliminate contamination entirely, there were made, in addition to the above, a number of cultures using

individual bacteroids which had been isolated by the pipette method of Barber ('14). Individual bacteroids obtained from *Blatta orientalis* were placed in hanging drops of peptone or ascitic fluid-bouillon. These bacteroids were charted and observed at frequent intervals. Ten hanging drops were made, the total number of bacteroids in these drops being about 100. No growth whatsoever of the bacteroids or of any other organism took place in these hanging drops. Single bacteria from a laboratory culture placed in control hanging drops multiplied readily. The results of the entire series of culture experiments on the three species of Blattidæ indicate that the bacteroids do not grow readily, if at all, on routine culture media.

It is thus seen that the culture studies of the various investigators have yielded conflicting results. The three earlier investigators, Blochmann, Krassilschtschik and Forbes, did not succeed in growing the bacteroids. Mercier obtained from *Blatta orientalis* an organism closely resembling a number of common contaminators. If the bacteroids could be grown on routine media as readily as *Bacillus cuenoti*, it should be possible to obtain this organism in all cultures inoculated with bacteroids from any stage in the life history of the roach, *i.e.*, from the fat-body, egg or embryo. The work of Javelly and the writer, in which many sterile cultures were obtained, and in which *Bacillus cuenoti* was not found, would seem to indicate that Mercier's organism was itself a contaminator. The results of Javelly and the writer further agree in indicating that the bacteroids of *Blatella germanica* as well as those of *Blatta orientalis* do not grow readily, if at all, in routine culture media.

In Glaser's work with *Periplaneta americana* and *Parcoblatta virginica*, he obtained from each species a spirillum, these two spirilla differing from each other only in minor cultural characteristics. They differ markedly, however, in both morphology and cultural characteristics from Mercier's *Bacillus cuenoti*. Glaser inoculated his cultures with bacteroids obtained from the fat-body of adults. The abdomen was washed with alcohol or a mixture of alcohol and corrosive sublimate, and dissected with sterile instruments. The portions of fat-body containing

bacteriocytes were transferred directly to media and incubated. In the writer's experiments with *Periplaneta americana*, three tubes of bouillon were inoculated with bacteroids from three öothecæ, employing the technique described above. These three cultures remained sterile. A portion of the fat-body of a nymph was obtained by pulling apart the segments of the abdomen as described above, and a tube of bouillon inoculated. No growth occurred. In making a second transfer from this same nymph, the digestive tract was injured and contamination resulted. No other cultures were made from this species. While the number of cultures made from *Periplaneta americana* was limited, the results are seen to be in perfect agreement with those obtained by both Javelly and the writer for *Blatella* and *Blatta*. If the spirillum obtained by Glaser from *Periplaneta americana*, and which grows so readily in a great variety of routine media, actually represents the cultural form of the bacteroids of this species, it is difficult to account for the consistently sterile cultures obtained by the writer.

It was not stated by Glaser whether his technique yielded pure cultures of the spirilla in all cases, or whether contaminations were encountered. The subject of contamination was not discussed, either as concerns organisms in the cultures other than the spirilla or as to the possibility of the spirilla themselves being organisms other than the bacteroids. In view of the conflicting results of various investigators and the small size and filthy habits of the cockroach, the matter of possible contamination would seem to be a paramount consideration. In Mercier's work the flaming of the oötheca did not prevent contamination, and indeed, from his control experiments in which he obtained sterile cultures from the clear liquid bathing the eggs, it would appear that *Bacillus cuenoti* was obtained from within the egg. Javelly obtained contamination in cultures from the fat-body. In the writer's work, contamination was encountered in cultures from both oöthecæ and fat-body. It would seem impossible entirely to avoid contamination, except when employing single bacteroids isolated by the Barber or equivalent technique. The possibility of organisms other than

the bacteroids occurring within the tissues or body cavity of the roaches is not precluded, since all the writer's specimens of *Periplaneta americana* were heavily infected with a gregarine, *Diplocystis* sp., inhabiting the body cavity. Glaser did not mention any control experiments showing that the washing of the roach with alcohol or other reagents was successful in sterilizing the surface of the roach. It is conceivable that such reagent might fail to sterilize the areas where one segment overlaps another. In referring to the definite wanderings of the bacteroids in the body of the roaches and in the yolk of the egg, Glaser stated that the organisms in the species studied by him were motile. It is not clear whether motility of the bacteroids was observed directly, or whether the motility of the spirilla in cultures was referred to. In the writer's work many fresh preparations of the bacteroids obtained from five species of Blattidæ, including *Periplaneta americana*, were studied. No suggestion of motility was observed in any case. In view of the conflicting evidence and the technical difficulties involved, Glaser's evidence that the spirilla represent the bacteroids in culture would seem to be inadequate.

As concerns *Blatella germanica* and *Blatta orientalis*, the work of Javelly and the writer indicate quite definitely that the bacteroids do not grow on routine culture media, and that *Bacillus cuenoti* of Mercier is a contaminator. The entirely negative results of the writer in the culture of the bacteroids of *Periplaneta americana*, combined with the evidence from the other species, would seem to furnish strong presumptive evidence that the spirillum obtained by Glaser does not represent the cultural form of the bacteroids of this species.

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